Guidance for Industry

Role of HIV Drug Resistance Testing in Antiretroviral Drug Development

DRAFT GUIDANCE

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For questions on the content of the draft document contact (CDER) Jeffrey Murray or Kimberly Struble at 301-827-2330.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

November 2004
Clinical Antimicrobial

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Table Of Contents

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- *Clearly explain each issue/concern and, when appropriate, include a proposed revision and the rationale/justification for the proposed change.*
- *Identify specific comments by line number(s); use the PDF version of the document, whenever possible.*

I. INTRODUCTION

This guidance is intended to assist sponsors in the clinical development of drugs for the treatment of human immunodeficiency virus (HIV) infection. Specifically, this guidance addresses the Agency's current thinking regarding the role of HIV resistance testing during antiretroviral drug development and marketing. The guidance is also intended to serve as a focus for continued discussions among the Division of Antiviral Drug Products (DAVDP), pharmaceutical sponsors, the academic community, and the public.

This guidance focuses on resistance to antiretroviral agents as manifested by mutations in the HIV viral genome that result in reduced phenotypic susceptibility to a given drug product. While mechanisms of cellular resistance to antiretrovirals exist, a discussion of them is beyond the

[1] This guidance has been prepared by the Division of Antiviral Drug Products in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

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scope of this document. In addition, loss of susceptibility to drugs is highlighted, rather than hypersusceptibility. Finally, because the field of HIV resistance is evolving, we intend to revise the guidance as new information accumulates.

A related guidance entitled *Antiretroviral Drugs Using Plasma HIV RNA Measurements — Clinical Considerations for Accelerated and Traditional Approval* [2] offers guidance on trial design and endpoints in phase 3 antiretroviral drug development.

This guidance does not imply that one type of resistance testing is more useful than another type of resistance testing in the clinical management of HIV. This guidance does not address any strategies for clinical management of individual patients with HIV, but addresses how serial assessments of both genotype and phenotype are useful in antiretroviral drug development. For characterizing the utility of an antiretroviral drug, both phenotypic and genotypic resistance testing have strengths and limitations as discussed in this document. In addition, this guidance does not address the use of virtual phenotype data in drug development. Sponsors should discuss with the Division in advance any plans to incorporate virtual phenotype into trials.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The primary sources for this guidance document are (1) a two-day session of DAVDP's advisory committee, convened November 2-3, 1999, to address issues relating to HIV resistance testing; (2) the Division's experience with reviewing resistance data for antiretroviral drugs in new drug applications from 1999 to the present including subsequent analysis and presentation of resistance data to the DAVDP advisory committee; and (3) input from pharmaceutical sponsors and the HIV community. Presentations during the November 1999 advisory committee meeting included the following topics:

- performance characteristics of genotypic and phenotypic assays
- prevalence of resistance in antiretroviral naïve patients
- the ability of baseline resistance testing to predict subsequent virologic response
- clinical factors that might influence the results of resistance testing

[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance page at http://www.fda.gov/cder/guidance/index.htm.

Summaries of presentations at this meeting have been published in *Antiviral Therapy* (Laessig et al., 2000).

III. HIV RESISTANCE TESTING — GENERAL

Due to its high rate of replication (10^9-10^{10} virions/person/day) and error prone polymerase, HIV can easily develop mutations that alter susceptibility to antiretroviral drugs. As a result, the emergence of resistance to one or more antiretroviral drugs is one of the more common reasons for therapeutic failure in the treatment of HIV. In addition, the emergence of resistance to one antiretroviral drug sometimes confers a reduction in or a loss of susceptibility to other or all drugs of the same class.

The application of laboratory technologies such as gene amplification, automated nucleic acid sequencing, nucleic acid hybridization, and availability of recombinant viruses for testing phenotypic susceptibility have permitted advances in HIV resistance testing. Many clinicians and investigators are currently using these technologies in the clinical management of HIV. However, only one HIV resistance assay has been FDA approved, and performance characteristics (e.g., sensitivity, specificity, and reproducibility) for many of the assays in investigational use have not been fully established. In addition, the clinical significance of many mutations or mutational patterns has not been defined completely for many antiretroviral drugs. Likewise, the quantitative relationship between reductions of in vitro susceptibility and loss of clinical activity has not been established for most drugs. Consequently, many of the current package inserts are deficient in the amount and type of resistance data describing the utility of a drug in the setting of resistance or reduced susceptibility.

Despite limitations of resistance assays and their interpretation, several randomized controlled studies have demonstrated that virologic outcome, at least over the short-term, may be improved when genotypic or phenotypic data are used to guide choice of drug regimens in patients with loss of virologic response to prior regimens (Baxter et al. 2000; Cohen et al. 2002; Durant et al. 1999; Melnick et al., 2000; Meynard et al, 2000; Tural et al., 2002). The Division believes that characterization of resistance/cross-resistance should be a part of antiretroviral drug development so that clinically relevant information will be available at the time of approval. An efficient way to accomplish these goals is to include resistance testing in all phases of drug development with an emphasis on earlier stages of development. As discussed below, assessment of resistance should not be delayed until phase 3 or postapproval. We recommend that, prior to or during phase 1 and 2 studies, investigators begin assessing the potential of a drug to select resistant viruses and the drug's activity against HIV isolates resistant to other antiretroviral agents. During early development, a wide range of doses is evaluated and pharmacokinetic data are collected, providing information to investigate the relationship between drug exposure and resistance.

Optimally, a comprehensive evaluation of a new drug's resistance and cross-resistance profile will promote more rational use of antiretroviral drug combinations in the future. Although this

document focuses on characterization of resistance/cross-resistance during drug development, we recommend that these principles also be applied to the currently marketed antiretroviral agents.

IV. NONCLINICAL STUDIES

In vitro studies provide useful information for the design of in vivo studies and can be predictive of the development of resistant viruses in vivo. The nonclinical virology section of this document identifies studies relevant to resistance issues in the development of antiretroviral drugs for the treatment of HIV infection. The Division is also developing a nonclinical guidance document that will elaborate on general nonclinical studies for antiviral drugs.

The Division prefers that the nonclinical studies (i.e., mechanism of action, antiviral activity in vitro, cytotoxicity/therapeutic index, and effects of serum protein binding on antiviral activity) be examined before the initiation of phase 1 clinical studies. In vitro drug combination activity studies for drugs that are used in clinical trials should be completed prior to initiation of those trials. In vitro selection of resistant HIV-1 variants, the phenotypic and genotypic characterization of resistance viruses, and cross-resistance analyses should be examined prior to initiation of clinical studies in HIV-infected patients. We recommend that sponsors be consistent in the assay used for any particular analysis or measurement in studies.

A. Mechanism of Action

A well-characterized mechanism of action for a new antiretroviral drug can provide insight into the regions of the HIV genome where mutations that confer resistance may develop. These regions are not limited to the site of action (viral-encoded target) of the investigational drug and can include the enzyme substrate(s) (e.g., Gag and Gag-Pol cleavage sites for protease inhibitors) or another viral-encoded protein(s) existing in a quaternary complex with the target protein (e.g., gp120 and gp41). Any metabolite that exerts inhibitory activity should be delineated and its specificity for the target shown. For example, reverse transcriptase (RT) inhibitors should show selectivity for RT over cellular DNA and RNA polymerases.

B. Antiviral Activity In Vitro

The in vitro antiviral activity of a compound indicates that it effectively inhibits replication and forms the basis for defining phenotypic resistance (detected by reductions in susceptibility to the investigational drug, see below). The concentration of an investigational drug required to inhibit virus replication by 50 percent (IC_{50}) should be determined. The use of the IC_{50} value for determining shifts in susceptibility is preferred because it can be determined with greater precision than an IC_{90} or IC_{95} value. A well-characterized wild-type HIV laboratory strain grown in peripheral blood mononuclear cells (PBMCs) should serve as a reference standard.

The antiviral activity of drugs can vary greatly due to, for example, genetic variation in isolates, host cell type, multiplicity of infection assay used for measurement of virus replication. Because of genetic variation, determination of antiviral activity against a broad spectrum of viruses (50-

100 well-characterized laboratory strains and clinical isolates of HIV) is recommended. Antiviral activity should be assessed in multiple clade B and non-clade B isolates of T-cell tropic HIV-1, monocyte/macrophage tropic strains, HIV-2, well-characterized drug-resistant laboratory strains and clinical isolates, real-time isolates, and isolates representative of the virus population where clinical trials are to be conducted. This information will provide insight into the breadth of antiviral activity.

Protein binding of an antiviral drug to serum/plasma proteins may result in reduced antiviral activity. The effects of 45-50% human serum and/or human plasma plus α-acidic glycoprotein on the in vitro antiviral activity of the investigational drug should be evaluated for multiple laboratory and clinical isolates, and serum-adjusted IC_{50} values should be determined (see section V.D.2).

C. In Vitro Selection of Drug Resistant HIV-1 Variants

Selection of resistant viruses in cell culture may indicate whether development of resistance to a drug is likely to require a few (1-2) or many (> 2) mutations. The ability of an investigational drug to select HIV-1 variants with reduced drug susceptibility (phenotypic resistance) should be determined in cell culture systems. Selection of variants resistant to the investigational drug should be repeated several times to determine if the same or different patterns of resistance mutations develop.

Two basic methods have been developed to identify mutations conferring a reduction in susceptibility to a drug.

- In the first method, a high initial virus inoculum is propagated for several passages at a fixed drug concentration, using multiple cultures to test different concentrations. This approach is useful in identifying drugs for which there is a low genetic barrier to resistance.

- In the second method, virus is passaged in the presence of increasing drug concentrations starting at twice the IC_{50} value for the parent virus. Virus production is monitored to detect the outgrowth of resistant virus that is characterized with respect to genotype and phenotype.

1. Genotype

Mutations responsible for reductions in susceptibility to a drug can be identified by DNA sequence analysis of the relevant portions of the virus genome. The complete coding sequence of the gene for the target protein should be determined in the early stages of characterization of mutations associated with reduced drug susceptibility. Once mutations are identified, their ability to confer phenotypic resistance should be evaluated in a recombinant virus system (e.g., by using site-directed mutagenesis or polymerase chain reaction (PCR) amplification of relevant portions of the virus genome to introduce these mutations into a standard laboratory HIV genetic background). Recombinant virus should then be tested in vitro for drug susceptibility. Shifts in drug susceptibility (fold-

increases in IC_{50} value) for recombinant virus relative to wild-type should be determined (see section IV.E Characterization of Genotypic and Phenotypic Assays).

2. Phenotype

Drug susceptibility (IC_{50} values) for resistant variants and the fold change in IC_{50} values relative to the parent virus should be determined (see section IV.E Characterization of Phenotypic and Genotypic Assays). Drug resistant variants exhibit a statistically significant increase in the IC_{50}.

A number of drugs targeting the CCR5 and CXCR4 chemokine co-receptors are being developed. A potential concern with CCR5 inhibitors is that resistance may develop by switching to the CXCR4 co-receptor. The evolution of HIV to a CXCR4-utilizing virus has been proposed to result in a more virulent virus. Therefore, sponsors should monitor co-receptor switching in drug selection experiments and in clinical trials.

D. Cross–Resistance

HIV variants resistant to one drug in a class of antiretroviral agents may be resistant to another drug in the same class. Recombinant viruses containing drug resistance associated mutations to an investigational drug should be tested for susceptibility to approved and investigational drugs of the same class. Conversely, laboratory strains and 10-30 well-characterized clinical isolates containing resistance-associated mutations for each of the approved and investigational members of the same class should be tested for susceptibility to the investigational drug. Clinical isolates should be representative of the breadth of diverse mutations and combinations of mutations known to confer reduced susceptibility. Cross-resistance is not necessarily reciprocal, so it is important to evaluate both possibilities.

E. Characterization of Genotypic and Phenotypic Assays

Well-characterized genotypic and phenotypic assays provide the basis for the analysis of the emergence of resistant virus during the development of investigational drugs.

1. Genotypic Assays

The performance characteristics of genotypic assays should be described, including elaboration of the following characteristics:

- minimum plasma viral RNA level
- purification methodology for viral nucleic acids
- amplification methodology and primers
- PCR controls
- clade differences
- nucleic acid sequencing methodology

- description of sequencing primers
- range of mutant/wild-type ratios detectable
- interpretation criteria for mutant scoring

The entire coding sequence of the gene for the target protein should be determined in the early stages of analysis of resistant variants. Once the major mutations leading to resistance are identified, only the relevant portions of the genome need to be sequenced. The pattern of mutations leading to resistance of an investigational drug should be documented and compared with the pattern of mutations of other drugs in the same class.

Reporting the details of methodologies is important. The sponsor should identify sequencing primers and state how many bases from them can be read accurately. Sponsors should also define the sensitivity of the genotypic assay used for detecting minority viral subpopulations.[3]

2. Phenotypic Assays

The performance characteristics (accuracy, precision, limits of detection and quantification, specificity, linearity, range, robustness, stability) of an investigational phenotypic assay should be well documented. The sources of viruses (blood, plasma, etc.), their storage and stability, and cell culture procedures should be described. For definitions on assay validation, please refer to FDA's guidance for industry entitled *Bioanalytical Methods Validation*. An additional reference is ICH *Q2A Text on Validation of Analytic Procedures*. Sponsors are encouraged to use an assay that has been previously characterized and validated.

The utility of a phenotypic assay will depend upon its sensitivity (i.e., its ability to measure shifts in susceptibility (fold-changes) in comparison to baseline clinical isolates) and on the investigational drug's pharmacokinetics. To detect clinically relevant breakpoints, drugs for which the plasma levels are close to the IC_{50} value can call for an assay with greater sensitivity than would be sufficient for drugs maintaining plasma levels far in excess of the IC_{50} value. Shifts in susceptibility for a clinical isolate are measured by determining the IC_{50} values for the isolate and a wild-type standard virus done under the same conditions and at the same time. This provides for absolute comparisons between assays. Readout of phenotypic assays can be detected with any standard virus assay, such as p24, viral RNA, RT assay, MTT cytotoxic assay, reporter gene expression.

[3] A draft guidance with information on genotypic assay validation entitled *Premarket Notifications [510(k)s] for In Vitro HIV Drug Resistance Genotype Assays* issued in August 2001 (available on the Internet at http://www.fda.gov/cber/guidelines.htm). Once finalized, it will represent the Agency's perspective on this issue.

V. CLINICAL: USE OF RESISTANCE TESTING IN CLINICAL PHASES OF DRUG DEVELOPMENT

Prior to advances in resistance testing technologies, resistance/cross-resistance data were often not obtained until late in drug development or in the postmarketing period. However, given the current availability of resistance testing in clinical practice and the need to disseminate to health care providers information about an antiretroviral drug's resistance profile, comprehensive resistance testing should be undertaken in all phases of drug development. Crucial decisions in protocol design and drug development plans hinge on resistance and cross-resistance data and are discussed in this document.

In vitro resistance and cross-resistance studies of an investigational drug help focus the scope of drug development. For example, drugs that exhibit extensive cross-resistance with approved drugs of the same class are unlikely to be suitable for studies in treatment experienced patients harboring resistant isolates to that class. Conversely, for drugs that demonstrate a nonoverlapping or unique resistance profile, the Division strongly encourages sponsors to develop clinical protocols studying treatment experienced individuals.

Epidemiological data suggest that transmission of drug resistant HIV is on the rise, meaning that one cannot assume that treatment naïve patients harbor wild-type virus (Little et al., 2002). In this regard, the Division strongly recommends that samples for baseline resistance testing (preferably for both genotype and phenotype) be collected on all HIV-infected participants in multiple-dose studies. Knowledge of genotype/phenotype at baseline can aid in the interpretation of unexpected antiviral responses, particularly in smaller dose-ranging studies.

Based on the principles summarized above, the goals of resistance testing in clinical trials are threefold:

1. To determine the effect of the drug of interest on evolution of the virus, as measured using genotypic and phenotypic testing at baseline and follow-up and as assessed by evaluating virologic response to an initial or subsequent regimen
2. To determine the susceptibility and virologic response of the drug of interest in patients who have non-wild-type virus at baseline
3. To determine baseline genotypic and phenotypic determinants of virologic success or failure

A. General Considerations

Data from nonclinical studies and phase 1 and 2 clinical trials should provide a preliminary idea of the genotypic mutations that confer reduced drug susceptibility and a lack or loss of virologic response. Phase 3 trial designs should incorporate this information and expand on it, thereby aiming to further characterize drug resistance.

The Division strongly recommends resistance testing in all phases of development and in most cases, as soon as the drug is introduced into HIV-infected patients. In general, the type of

information collected and the types of analyses conducted should be the same for all phases of development. Whenever possible, resistance analyses should be prospectively defined. However, since it is not possible to define *a priori* key mutations or susceptibility breakpoints, retrospective analyses can provide important information in characterizing resistance and cross-resistance. The following sections provide recommendations on the type of resistance data that should be collected during development and the types of analyses that should be conducted. Issues relating to a specific phase of development will be highlighted separately. Appendix A provides a template for submitting HIV resistance data. Information about the specific assays and mutational algorithms used in protocols should also be provided in advance to the Division.

B. Data Collection

To characterize drug resistance during development, sponsors are strongly encouraged to collect the following information:

- Baseline phenotype and genotype on all study participants.

  The reasons for obtaining baseline samples for phenotype and genotype on all clinical trial participants are twofold. First, the prevalence and rate of transmission of drug resistant virus is increasing (Little et al., 2002), and may continue to increase, as the HIV population becomes more treatment experienced. Second, collection of baseline data provides an opportunity to examine the relationship between genotype/phenotype and virologic response to drug. Use of resistance testing in study protocols may help in choosing appropriate combination regimens for treatment experienced patients (see section V.C.4 for further details).

- Post-baseline phenotype and genotype on all study participants who demonstrate a lack or loss of virologic response during the trial.

  Collection of these data provides for the determination of mutations that may contribute to reduced drug susceptibility. Phenotypic and genotypic testing should be performed on all patients at the time that a lack or loss of virologic response is established. It is important to collect samples for resistance testing when subjects are still on study drug, or as soon as possible if study drugs are discontinued. Studies have shown that wild-type virus may outgrow resistant HIV viral strains in the absence of selective drug pressure (Devereaux et al., 1999; Halfon et al., 2003). In addition, continuation of resistance monitoring on subsequent regimens is important, where applicable.

C. Types of Analyses

Several types of resistance analyses can be used to characterize a drug's resistance profile. Some analyses are possible only when larger datasets are available. In phase 3, clinical trial datasets may be sufficiently large to study the effect that mutations confer upon drug susceptibility and outcome. Pooling data from several trials (provided the study populations, endpoints, and assays

are similar) can be appropriate, but should be discussed in advance with the Division. To facilitate pooling data, sponsors should attempt to use similar, if not identical, assays throughout the course of drug development. Whenever possible, resistance analyses should be prospectively defined, with the caveat that it is not always possible to define *a priori* key mutations or susceptibility breakpoints. In some cases, retrospective analyses can provide important information in characterizing resistance and cross-resistance.

Because of a large number of potential comparisons, statistical testing can be problematic for analyses of resistance testing and outcome. Sponsors are encouraged to submit resistance analysis plans to the Division in advance.

1. Baseline Genotype and Virologic Response

Analyses should be conducted to evaluate HIV RNA response according to the presence and absence of baseline mutations. These analyses will help assess the association between a specific mutation or mutational pattern and virologic response rates. For example, for a new nucleoside analogue, virologic response rates would be determined for patients with and without clinically relevant mutations associated with resistance to other nucleoside analogs. Analyses of virologic outcome by baseline genotype should be based on the as-treated population. Subjects who discontinue study treatment while suppressed or who discontinue study treatment before confirmed suppression for adverse event, noncompliance, protocol violation, pregnancy, or withdrawal of consent can be censored. Rules for censoring subjects who appear to have a virologic response prior to discontinuation should be discussed with the Division. The virologic response parameters used in these analyses are (1) proportion, < 400 copies/mL, (2) proportion, < 50 copies/mL, and (3) mean change from baseline at the protocol-specified timepoints. Sponsors are requested to provide analyses on all three endpoints and should discuss endpoints with the Division in advance. All subjects should be included in the dataset until the time of censoring. The datasets should include variables for reasons for censoring subjects. Please refer to Appendix A for further details. Table 1 below is one example of virologic response rates for a hypothetical nucleoside analog by the presence or absence of zidovudine-associated mutations at baseline.

Table 1. HIV RNA Response by Baseline RT Mutations at Endpoint

Baseline RTI Mutations*	Virologic Response = Proportion < 400 copies/mL			
	MUTATION PRESENT		MUTATION ABSENT	
	Drug X (n =)	Control (n =)	Drug X (n =)	Control (n =)
Any RTI mutation	50%	6%	80%	11%
M41L	27%	6%	79%	7%
D67N	65%	3%	63%	2%
K70R	72%	3%	54%	2%
L210W	17%	6%	72%	5%
T215Y/F	38%	3%	80%	7%
K219Q/E/N	60%	9%	58%	7%

* Patients included in these subgroups may have other RTI mutations or mutations in addition to the baseline mutations listed.

Table 1 shows response rates according to the presence or absence of specific mutations. In clinical isolates, however, mutations often occur in patterns, some of which are considered primary and others compensatory or accessory. Exploratory analyses should be conducted to define sets of mutational patterns with the largest impact on subsequent response rates.

For some drugs, defining specific mutational patterns that best correlate with a reduction in treatment response is difficult. In these cases, another approach is to investigate the number of baseline mutations that affects overall response. For example, the response rate (< 400 copies/mL) in subjects with < 5 protease inhibitor (PI) -associated mutations at baseline may be 80% compared to 25% if > 5 PI-associated mutations are present at baseline. For some drugs, the number and types of mutations may be important for overall clinical response. Therefore, we recommend sponsors conduct analyses as suggested in tables 2A and 2B. Sponsors should discuss in advance with the Division the specific mutations included in the overall number for the suggested analyses. Additional exploratory analyses may be recommended to further investigate the impact of certain mutation(s) on virologic response.

Table 2A. HIV RNA Response by Number of Baseline Mutations at Endpoint

# baseline mutations[1,2]	**Proportion < 400 copies/mL**	
	Drug X (n =)	**Control (n =)**
No mutations	80%	11%
Any mutations	50%	0
1–2 mutations	66%	4%
3–4 mutations	44%	4%
≥ 4 mutations	35%	3%
≥ 3 mutations + mutation at x or y	21%	1%
≥ 3 mutations / No mutation at x or y	67%	7%

[1] Patients included in these subgroups may have other thymidine analogue mutations or mutations in addition to the baseline thymidine analogue mutations listed.

[2] Thymidine analogue mutations include any change at D67, K70, L210, T215, K219.

Please note the number of PI mutations (4) used in the analysis for table 2B is for illustrative purposes. The number of mutations that affect virologic response rates vary for each drug; therefore the number and type of mutations used for analyses of HIV RNA response should be discussed with the Division.

Table 2B. HIV RNA Response by Number and Type of Mutations at Endpoint

Baseline Primary PI Mutation[1,2]	Number of Mutations			
	Drug X		Control	
	< 4 PI Mutations (n =)	≥ 4 PI Mutations (n =)	< 4 PI Mutations (n =)	≥ 4 PI Mutations (n =)
30	80% (4/5)	80% (8/10)	67% (4/6)	60% (6/10)
36	100% (6/6)	50% (6/12)	76% (19/25)	24% (4/17)
46	77% (24/31)	32% (7/22)	38% (3/8)	26% (5/19)
54	75% (3/4)	24% (6/25)	67% (4/6)	31% (9/29)
73	67% (4/6)	34% (10/29)	75% (6/8)	50% (7/14)
77	77% (17/22)	30% (3/10)	79% (19/24)	42% (5/12)
82	53% (7/13)	31% (8/26)	50% (7/14)	31% (9/29)
88	75% (6/8)	44% (15/34)	58% (7/12)	25% (2/8)
90	70% (7/10)	40% (14/35)	56% (5/9)	9% (3/32)

[1] The isolates include but are not limited to the indicated mutation. Most patients had > 1 PI resistance-associated mutation at baseline.

[2] Primary mutations include any change at D30, V32, M36, M46, I47, G48, I50, I54, A71, G73, V77, V82, I84, N88, and L90.

Note: Results should be interpreted with caution because the subgroups were small

2. Development of HIV Mutations

The Division strongly recommends that genotypic testing be performed on all patients who meet the definition of a lack or loss of virologic response, preferably while on study drug or as soon as possible after discontinuation of study drug. Studies have shown that wild-type virus may outgrow resistant HIV strains in the absence of selective drug pressure. For this reason, it can be useful to collect and store samples for resistance testing at the same timepoints that HIV RNA testing is done. These samples can provide important information on the development of resistance, especially for drugs that may have more than one possible resistance pathway.

The proportion of subjects who develop any NRTI (nucleoside analogue reverse transcriptase inhibitor)-, NNRTI (nonnucleoside reverse transcriptase inhibitor)-, or PI-associated mutation and the time to development of these mutations should be presented. Both primary and secondary mutations should be evaluated. For example, for subjects receiving a new PI, it is important to evaluate the development of primary and secondary PI mutations along with any other changes in the PR (protease) and RT gene, when applicable. It is also important to assess the genotypic basis of drug susceptibility changes attributable to extragenic sites, such as the protease cleavage sites.

3. Baseline Phenotype and Virologic Response

Analyses should also be conducted to define the decrease in phenotypic susceptibility that adversely affects virologic response (i.e., susceptibility break-points). It is important to evaluate potential susceptibility break-points for which no response or reduced response rates are

anticipated. In addition, determination of baseline susceptibility to other drugs within the investigational drug's class is important. Agreement on susceptibility breakpoints for most antiretroviral agents is limited; therefore, the median-fold change in susceptibility can be used as a breakpoint. Alternatively, other breakpoints could be proposed but should be discussed with the Division in advance. Examples of both analyses are presented in tables 3 and 4 below.

Table 3. HIV RNA Response (< 400 copies/mL) to Drug X by Baseline Susceptibility

Baseline Drug X Susceptibility	Drug X (n =) HIV RNA < 400 copies/mL
≤ 1	78%
> 1 and ≤ 2	55%
> 2 and ≤ 3	56%
> 3 and ≤ 4	7%
≤ 4	61%
> 4	14%

Table 4. HIV RNA Response (< 400 copies/mL) to Drug X by Median Baseline NNRTI Susceptibility

Baseline NNRTI Susceptibility	Drug X (n =)	Control (n =)
Drug X (median 2.1)		
< 2.1	72%	41%
≥ 2.1	42%	21%
NNRTI A (median 3.7)		
< 3.7	81%	47%
≥ 3.7	33%	13%
NNRTI B (median 41.7)		
< 41.7	45%	33%
≥ 41.7	63%	2%
NNRTI C (median 1.8)		
< 1.8	58%	46%
≥ 1.8	53%	23%

4. Genotypic – Phenotypic Correlations: Changes in Susceptibility from Baseline

Assessing changes in susceptibility over time on treatment is an important factor in the characterization of a drug's resistance profile. For the group of subjects who meet the definition of a lack or loss of virologic response, evaluation of the mean and median-fold changes in susceptibility from baseline for the investigational drug alone and other approved drugs from

both inside and outside the same class is important. In addition, analyses should be conducted on groups of subjects who develop a particular new mutation(s) during treatment, and the median-fold change in susceptibility from baseline should be presented. Table 5 is an example of how to display data from this analysis. Efforts should also be made to define relationships between genotype and phenotype.

Table 5. Development of Mutations and Median Change in Susceptibility from Baseline

Patients Developing New Entry Inhibitor Mutations	N	Median-fold Change in Susceptibility from Baseline	
		Drug X	Control
Mutation A			
None by week 24		2.1	3.2
Yes by week 24		2.5	5.5
Mutation B			
None by week 24		1.6	2.6
Yes by week 24		1.2	2.2
All Patients Analyzed		1.9	3.2

5. Cross-Resistance

The evaluation of cross-resistance with other drugs in the same class is important. Characterization of cross-resistance of a drug provides important and valuable information to patients and health care providers because these data provide for a more informed selection of subsequent antiretroviral regimens. Evaluation of the effect of the investigational drug on subsequent use of other drugs and how previous treatment with other drugs may affect the response to the investigational drug is essential in drug development. The former can be accomplished by designing rollover studies evaluating virologic response rates in subjects discontinuing study drug in clinical trials.

Phase 3 trials should incorporate prospective rollover designs to provide for assessment of virologic responses in study subjects administered subsequent antiretroviral regimens. When possible, the design of the rollover study should include a randomized control. Every effort should be made to capture as much information as possible from the original studies. Resistance testing can be used to assess the genotype and phenotype of antiretroviral experienced patients that predict success or failure after exposure to previous therapies. This testing can involve longer follow-up of study subjects, perhaps continuing into the postmarketing period.

6. Additional Analyses

In addition to the analyses suggested above, sponsors should consider conducting the following investigations.

- Where appropriate, exposure-response analyses, which will require obtaining pharmacokinetic information from some proportion of study patients. The goal of such analyses is to determine which drug exposure measures (AUC, C_{trough}, etc.) are relevant to a given virologic outcome.
- Evaluation of drug exposure measurements in individual patients while appropriate drug levels are maintained. These evaluations help to distinguish true virologic failure from treatment failure due to decreased bioavailability or other reasons.
- Pharmacogenetic analyses to determine genetic factors that may be involved in virologic response (e.g., for co-receptor inhibitors that target a host receptor, it is important to understand if genetic differences in the receptor affect response).

D. Other Considerations

1. Role for Supporting Initial Activity/Dose-Finding Studies

Primary objectives of initial studies in HIV-infected patients are to establish that the new investigational agent has anti-HIV activity, to determine the magnitude of that effect, and to determine the most active dose(s) that can be taken forward in larger studies. Often, studies that incorporate short periods of monotherapy (e.g., $\leq$ 2 weeks) or functional monotherapy (when a drug is added to a failing but stable regimen) have been helpful in accomplishing these objectives. Compared to combination studies, such protocols can more clearly delineate the effect of the drug of interest. However, resistant viruses sometimes emerge rapidly for certain drugs, such that periods of monotherapy can jeopardize a participant's future therapeutic options. Thus, some drugs are not candidates for use in monotherapy trials, including trials of very short duration. Drugs exhibiting a low genetic barrier (such as those in which a single mutation is easily selected and able to confer large reductions in susceptibility to the new drug and other drugs of the same class) should probably not be studied as monotherapy, particularly in treatment naïve individuals. Data from in vitro resistance studies should be used to determine whether a new drug could safely be administered as a single agent for limited periods of time. Please refer to Appendix B: Criteria for Resistance-Conferring Mutations.

2. Dose-Finding Trials

Sponsors should collect baseline genotype/phenotype information in HIV-infected subjects who participate in pharmacokinetic/dose finding studies. Current evidence indicates that virologic response is better when drug levels can be maintained some increment above the serum-adjusted IC_{50} value (see section III page 5). Study subjects with baseline resistance mutations may require higher drug concentrations of the antiretroviral drug to achieve an antiviral response similar to the response observed in patients with wild-type virus. Patients with particular genotypes/phenotypes of interest should be prospectively identified for inclusion in dose-ranging studies.

3. Use of Resistance Data to Establish an Indication

The amount of evidence sufficient to characterize a drug's general resistance profile and the amount of data to support a specific efficacy claim against a particular resistant strain of HIV are not always the same. Well-controlled, randomized, prospective trials are preferred when attempting to develop a drug product to obtain a specific indication for use in a select group of patients with a particular resistance profile at baseline or antiretroviral treatment history. The amount of evidence sufficient to establish a claim of efficacy in a specific patient population will be substantial, and studies of small numbers of subjects are unlikely to accomplish this goal. Sponsors are encouraged to discuss their development plans with the Division in advance.

4. Use of Resistance Data for Study Enrollment Criteria, Background Regimen Selection, and Stratification Factors

As mentioned above, resistance testing at baseline can be helpful in selecting study participants with particular resistance profiles at baseline. For example, to evaluate the efficacy of a new NNRTI in patients who have failed previous NNRTI regimens, sponsors can elect to enroll subjects with confirmed genotypic and/or phenotypic NNRTI resistance. Phase 1 and 2 and nonclinical data can be used to define which mutation or mutations adversely affect response rate, so that in phase 3 trials, enrollment can be restricted to subjects without these mutations at baseline.

Some studies evaluate a new drug combined with an *optimized background*, meaning that the concomitantly administered antiretrovirals were chosen based on data from resistance testing. For trials that include an optimized background regimen, genotypic and/or phenotypic resistance testing is used to guide the selection of the background antiretroviral regimen. In addition, some studies have used genotypic and/or phenotypic susceptibility scores to quantify the number of drugs to which a participant may still be susceptible. Sponsors can also consider using an external expert committee to aid in the selection of background regimen, especially for subjects with limited therapeutic options.

Baseline resistance testing can also be used to stratify patients, providing more even distribution of participants with resistant isolates between treatment arms. Rationales for specific stratification factors should be discussed with the Division in advance.

5. Non-B Subtypes

Sponsors are also encouraged to evaluate baseline resistance data and response and the development of resistance in subjects with non-clade B viruses versus clade B viruses. Since many sponsors are conducting global development plans and it is unknown if different HIV subtypes develop resistance via different pathways, these data will add to the overall characterization of an investigational drug's resistance profile (Hirsch et al., 2003).

E. Phase 4

Resistance should continue to be monitored and further described during postmarketing of an antiretroviral drug. As more drugs enter the market, additional cross-resistance studies will help to further characterize the cross-resistance profile between new and existing antiretroviral agents.

VI. SUMMARY

The goal of this guidance is to stimulate the generation of more complete resistance data and analyses for antiretroviral drug products. These analyses provide an indication regarding which mutations are clinically relevant and have an impact on the therapeutic success of a given product. Such information could potentially be included in drug labeling to facilitate appropriate prescribing of products and to maximize the chance for therapeutic success.

The preceding sections of this guidance include recommendations for how and when to obtain HIV drug resistance information. Appendix A provides a template for submitting HIV resistance data. Appendix B outlines how the analyses of resistance data might be interpreted and how to determine whether a mutation confers resistance. The decision that any given mutation is clinically relevant and deserves inclusion in product labeling is determined during the course of an NDA review.

APPENDIX A: TEMPLATE FOR SUBMITTING HIV RESISTANCE DATA

For each study, datasets should be provided as SAS transport files containing the following information.

- The datasets should include one record (row) per patient per isolate (e.g., baseline, failure, and other timepoints).

- The data on all isolates should be in columns (with suggested column headings shown below). (Note: in the SAS transport files, column headings can be given abbreviated column names to fit the SAS format, but a description of column names should be provided to the reviewer in the submission).

- Genotypic data should be provided for (at a minimum) baseline isolates of all patients and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate.

- Phenotypic data should be provided for (at a minimum) baseline isolates of all patients and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate.

The specific criteria for defining virologic failures should be discussed with the Division. This "definition" of virologic failure is intended to be used ONLY for the identification of patients from whom isolates should be collected for resistance testing and is NOT intended be used as a clinical or virologic endpoint in outcome analyses. Isolates from patients with documented clinical progression should also be included

One dataset combines patient data, endpoint data, genotypic data, and phenotypic data. There are a number of ways datasets can be subdivided (i.e., by clinical study, baseline isolates, or virologic failure isolates), and the subdivisions should be discussed with the Division before submission.

INFORMATION TO INCLUDE WITH SUGGESTED COLUMN HEADINGS

A. Patient Data:

- Patient identification number
- Isolate (e.g., baseline, Week 24, Week 48, Discontinuation. Multiple isolates should be numbered, e.g., failure 1, failure 2.)
- Date of isolate
- Previous therapeutic agents where available
- Treatment group

B. Endpoint Data:

- HIV RNA (copies/mL) at baseline
- HIV RNA (copies/mL) at predefined timepoints (e.g., Week 24 and Week 48)
- HIV RNA (copies/mL) at time of loss of virologic response or discontinuation due to adverse event
- Endpoint assessments (e.g., mean log change in viral load from baseline)
- Other endpoint assessments (e.g., DAVG, mean log change in viral load from baseline)
- Please indicate if data were censored for reasons other than virologic failure (e.g., discontinuation due to adverse event)
- Outcome (i.e., responder, virologic failure, discontinuation while suppressed, discontinuation before achieving viral suppression)
- Reason for discontinuation (i.e., adverse event, pregnancy, etc.) or failure (i.e., never suppressed, rebound)
- HIV RNA (copies/mL) from additional timepoints can be included.

C. Genotypic Data: (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)

- Clade

- Genotype information for all the RT and protease and gp 160 (for agents targeting entry only), one amino acid per column with the wild-type (WT) amino acid as column heading. Changes from WT standard sequence indicated (i.e., blanks indicate no change).

- **Column with total number of PI mutations in patient isolate (for baseline and endpoint isolates)**
  PI mutations to be counted include changes at amino acid D30, V32, M46, G48, I50, I54, G73, V82, I84, N88, L90, and any additional amino acid changes in PR that are important/relevant to the study drug as shown in vitro or in clinical trials. Please discuss with the Division in advance for agreement on the total number of mutations.

- **Column with total number of NRTI mutations in patient isolate (for baseline and endpoint isolates)**
  RT mutations to be counted include changes at amino acid M41, E44, K65, D67, T69, K70, L74, Y115, V118, M184, L210, T215, and any additional amino acid changes in RT that are important/relevant to the study drug as shown in vitro or in clinical trials. Please discuss with the Division in advance for agreement on the total number of mutations.

- **Column with total number of NNRTI mutations in patient isolate (for baseline and endpoint isolates)**

RT mutations to be counted include changes at amino acid A98, L100, K103, V106, V108, Y181, Y188, G190, P225, M230, P236 and any additional amino acid changes in RT that are important/relevant to the study drug as shown in vitro or in clinical trials. Please discuss with the Division in advance for agreement on the total number of mutations.

Table A1 highlights how genotype information should be displayed, but does not include all the column headings suggested above.

Table A1. Example of Genotype Information Display

Patient #	Isolate	V-82	N-83	I-84	I-85	G-86	R-87	N-88	L-89	L-90	# PI Mutations
001	BL							S		M/L	2
001	WK48			V				S		M	3
002	BL	A/T		V				D		M	4
002	WK48	T		V						M	3
003	BL	T		V							2
004	BL			V						M	2

BL = baseline
WK48 = Week 48 for investigational drug

Protease cleavage sites: FOR PROTEASE INHIBITORS ONLY

- p2/NC protease cleavage site: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant
- NC/p1 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant
- p1/p6 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant

G. Phenotypic Data: (minimally for baseline isolates and endpoint isolates from virologic failures and discontinuation)

1. Information on the Investigational Drug

- Baseline IC_{50} value for investigational drug
- Baseline IC_{50} value of reference strain for investigational drug
- Fold resistant change of baseline IC_{50} value compared to IC_{50} value of reference strain of investigational drug
- IC_{50} value at time of endpoint assessment or failure for investigational drug

- Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for investigational drug
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for investigational drug

2. Information on Approved/Investigational Agent(s) in the Same Class

(Please first list agents in the same class in alphabetical order, then list agents with the same target protein in alphabetical order. Finally, list agents outside the drug class in alphabetical order.)

- Fold change in IC_{50} value of baseline compared to reference strain for all approved/investigational anti-HIV agent(s)
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved/investigational anti-HIV agent(s)
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved/investigational anti-HIV agent(s)

3. Information on Approved/Investigational Agent(s) Outside the Investigational Drug's Class With Same Target Protein (e.g., NRITS and NNRTIS)

- Fold change in IC_{50} value of baseline compared to reference strain for approved/investigational agent(s) outside the investigational drug's class
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved/investigational agent(s) outside the investigational drug's class
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved/investigational agent(s) outside the investigational drug's class

4. Information on Other Antiretroviral Agents in the Regimen

- Fold change in IC_{50} value of baseline compared to reference strain for other antiretroviral agents in the regimen, one column/agent
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for other antiretroviral agents in the regimen, one column/agent
- Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for other antiretroviral agents in the regimen, one column/agent

Table A2 highlights how phenotype information should be displayed, but does not include all the column headings suggested above.

Table A2. Example of Phenotype Information

	Drug X				Other Drugs in Same Drug Class*		Other Drugs Outside Drug Class*	
Sample	IC_{50} value Drug X	Ref strain IC_{50} value Drug X	Δ resis from ref Drug X	Δ resis from BL Drug X	Δ resis from ref Drug Y	Δ resis from BL Drug Y	Δ resis from ref Drug A	Δ resis from BL Drug A
Baseline								
Endpoint								

Drug X = candidate drug

Δ resis = fold resistance change, e.g.: $\frac{IC_{50} \text{ value of baseline sample with Drug X}}{IC_{50} \text{ value of reference strain with Drug X}}$

ref strain = reference strain (or WT)

Endpoint = predefined timepoint for endpoint assessment (e.g., week 24, week 48, failure or discontinuation)

*Note: The Δ resis from ref and Δ resis from BL should be included for all approved anti-HIV drugs

H. Co-receptor Usage (for all agents targeting entry)

- Co-receptor usage of baseline isolates. Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column
- Baseline R5 assay value
- Baseline X4 assay value
- Co-receptor usage of virologic failures and end of study isolates (on therapy). Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column
- R5 assay value at failure/end of study
- X4 assay value at failure/end of study

APPENDIX B: GENETIC THRESHOLD FOR RESISTANCE

The genetic threshold for resistance can arbitrarily be divided into low and high categories and may vary as a function of drug concentration. A product with a low genetic threshold may select for resistance with only one or two mutations. In contrast, a product with a high genetic threshold may require several mutations to result in viral strains with reduced susceptibility. Sponsors should assess the development of resistance in vitro over the concentration range spanning the anticipated in vivo concentration. These distinctions were used to develop the following factors to consider for products with low or high genetic thresholds.

A. Low genetic threshold (≤ 2 mutations required for reduced susceptibility)

In vitro evidence

1. Single or double mutations appear after selection for a limited number of passages in cell culture.
2. Insertion of the mutation(s) by site-directed mutagenesis into standard laboratory strains yields strains with reduced susceptibility.
3. A mutation occurs at a significant frequency in clinical isolates, and the clinical isolates with the mutation(s) demonstrate reduced susceptibility.

Clinical evidence

1. Mutation(s) are detected at the time of virologic rebound in a significant number of patients.
2. There is suboptimal clinical response when the mutation(s) is present at baseline.

B. High genetic threshold (> 2 mutations required for reduced susceptibility)

In vitro evidence

1. Multiple mutations appear after serial passages of HIV-1 in cell culture in the presence of increasing concentrations of the agent.
2. Insertion of the mutation(s) by site-directed mutagenesis into standard laboratory strains yields strains with reduced susceptibility.
3. Clinical isolates with mutations demonstrate reduced susceptibility

Clinical evidence

1. Mutations detected at the time of virologic rebound (in a number of patients)
2. Loss of clinical response when mutations are present at baseline.

GLOSSARY

Key mutation: A treatment selected amino acid change that can cause a decrease in susceptibility to one or more antiretroviral agents of the same class.

Accessory or compensatory mutation: A mutation that by itself does not confer a decrease in susceptibility to antiretroviral agents. Accessory or compensatory mutations can augment key mutations and, perhaps, fitness mutations.

Polymorphism: Natural variation in the HIV-1 genome.

Fitness mutation: An amino acid change that compensates for the reduced virus growth resulting from a drug resistance-conferring mutation.

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